REMARKS

The foregoing amendments are presented to place the application in compliance with the sequence rules under 37 CFR 1.821-1.825.

Applicants have submitted a Sequence Listing in both paper and computer readable form as required by 37 C.F.R. 1.821(c) and (e). Amendments directing its entry into the specification have also been incorporated herein. The content of the paper and computer readable copies are the same and no new matter has been added.

In addition, the specification has been carefully reviewed and changes have been effected. In particular, the specification headings have been amended in conformance with U.S. practice. Other changes related to grammar and spelling have also been effected. We have also added sequence identifiers after each sequence in the specification and claims in accordance with the sequence rules.

Claims 1-8 have been amended to put the claims in better form under U.S. practice and in compliance with the sequence rules under 37 C.F.R. 1.821-1.825. Support for the claim amendments is readily apparent from the teachings of the specification and the original claims.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "**Version with markings to show changes made.**"

In view of the foregoing, it is believed that each requirement set forth in the Notice has been satisfied, and that the application is now in compliance with the sequence rules under 37

CFR 1.821-1.825. Accordingly, favorable examination on the merits is respectfully requested.

Respectfully submitted,

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A PEPTIDE HAVING AN AFFINITY FOR gp120

Version with Markings to Show Changes Made

BACKGROUND OF THE INVENTION

1. ~~TECHNICAL~~ FIELD OF THE INVENTION

This invention relates to a peptide which has an affinity for gp120, HIV (human immunodeficiency virus) envelope protein.

2. DESCRIPTION OF THE RELATED ~~BACKGROUND~~ ART

The therapy for HIV infection is usually chemotherapy, such as the nucleotide derivative AZT (3'-azido-3'-deoxythmidine). This AZT therapy or protease inhibitor, which was later developed, prolongs the life of HIV patients, but there are some problems, these are derived from the chemotherapy itself.

~~There~~ The problems are shown as follows: The first is chronic poisoning due to long term administration, the second is the appearance of an HIV virus resistant to the medicine during the therapy, the third is the appearance of malignant tumors in prolongation of the HIV patient's life, the fourth is that the recovery of the immune system can not be monitored, the fifth is that ~~the~~ there is not ~~being~~ a method to monitor treatment effect, etc. Since such chemotherapy is not basic therapy for HIV infections, most people anticipate the development of a vaccine.

Generally, the vaccine is an inactive treatment (in active vaccine) of a microbe of viruses; a weak activity virus which loses pathogenesis or a pseudo virus (live vaccine) which has no fatal effects to humans. However, although HIV itself is natively a weak

hybridization due to genetic engineering at the level of the protein as the Fab' itself. But, although with such neutralizing antibodies it is possible to inhibit HIV infection to the lymphocyte at laboratory level, an antibody that can be used practically has not yet been developed.

As mentioned above, chemotherapy has some problems; drug tolerance in the virus and side effects in the host, another idea to solve the problem of removing the virus from the body is by plasmapheresis. Although this method to remove the HIV virus by using a pore size membrane filter (smaller than virus size) for plasmapheresis has been definitely proposed it is not yet possible to make a uniform pore size membrane. It is also possible that the pores will become clogged during plasmapheresis resulting in the deterioration of the membrane due to pressure. As mentioned above there are many technical problems which have to be settled. So, a method to use CD4 derived from human lymphocyte having specific affinity to HIV, as absorbed carrier in column for plasmapheresis is also proposed. It cannot be used as a medical procedure because of the lost affinity due to decay by autoclave treatment. In addition, there are also methods using thermostable molecules, a high molecule polymer or color ligand as an affinity carrier to HIV. However, as these molecules do not originally have specific binding ability to HIV, they cannot be used because they bind to blood ingredients faster than to HIV.

In this way, aiming at the development of an HIV treatment medicine, research to produce a vaccine and ~~neutrizing~~ neutralizing antibody is flourishing, but useful medicine has not yet been developed.

The inventors paid attention to this present situation and developed a superior

peptide to have the same degree or more affinity for gp120 compared to antibodies and to be resistant to autoclave treatment, and have already made a patent application (Japanese Patent Application No. H 8-351474 and Japanese Patent Application No. 8-351475). This peptide basically consists of a three amino acid sequence, but from a study of the sequel, we found that an affinity to gp120 of this peptide deteriorated by number and a kind of the amino acid which ranged in it. So, we knew that we needed to develop a more stable peptide.

SUMMARY ~~DISCLOSURE~~ OF THE INVENTION

In view of the above, an object of this invention is to provide a novel peptide which has an affinity for gp120, HIV envelope protein, with excellent stability, and a variety of usabilities using the peptide.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph that shows result of EXAMPLE 8.

FIG. 2 is a photograph by electron microscope that shows the result of EXAMPLE 15.

DESCRIPTION OF THE PREFERRED EMBODIMENTS ~~BEST MODE FOR CARRYING OUT THE INVENTION~~

The No.1 peptide in this invention that could solve the above subject;

A peptide having an affinity to gp120 represented by formula (1):

H-A1-A2-A3-A4-A5-R (SEQ ID No. 1),

(in the formula,

H means hydrogen,

A1 is aspartic acid, lysine, valine , glutamic acid, glycine, ~~aspragine~~ asparagine, or tyrosine ~~residue~~ residue,

A2 is valine, aspartic acid, tryptophan, lysine, phenylalanine, isoleucine, leucine, or tyrosine residue,

A3 is lysine, valine, aspartic acid, arginine, alanine, or ~~Tryptophan~~ tryptophan residue,

A4 is alanine, tryptophan, or glycine residue

A5 is glycine, alanine, valine, leucine, isoleucine, serine, threonine, methionine, asparagine, glutamine, histidine, lysine, arginine, phenylalanine, tryptophan, proline, or tyrosine residue,

R is OH derived from carboxyl group or NH_2 derived from acid amide group).

Accordingly, the No. 1 peptide in this invention is a 5 amino acid sequence that was constituted by A1, A2, A3, A4 and A5 as described above, and all of the peptide including such amino acid sequences contained by the range of this invention. Thus, a peptide having an affinity to gp120 represented by

Formula(2): A1'-A2-A3-A4-A5-R (SEQ ID No. 2),

(in the formula,

A1' means aspartic acid, lysine, valine, glutamic acid,

glycine, ~~aspragine~~ asparagine, or tyrosine ~~reidue~~ residue, or polypeptide residue that an arbitrary amino acid stood in line in the N-terminal side from this amino acid, A2, A3, A4, A5 and R ~~bas~~ have the same meaning as above)

or

Formula (3): H-A1-A2-A3-A4-A5'-R (SEQ ID No. 3),

(in the formula,

A5' means glycine, alanine, valine, leucine, isoleucine,

threonine, methionine, asparagine, glutamine,

histidine, lysine, arginine, phenylalanine, tryptophan,

proline, or tyrosine residue, or polypeptide residue that an arbitrary amino acid stood in line in the C-terminal side of this amino acid, H, A1, A2, A3, A4 and R ~~bas~~ have the same meaning as the above)

is entirely one aspect of the present invention.

Then, the No. 2 peptide that could solve the above subject is;

a peptide having an affinity to gp120 represented by

Formula(4): H-a1-a2-a3-a4-a5-R (SEQ ID No. 4),

(In the formula,

H means hydrogen,

a1 is tyrosine, arginine, phenylalanine, glycine, tryptophan,

histidine, or ~~asparatic~~ aspartic acid ~~reidue,~~ residue

a2 is arginine, tyrosine, tryptophan, alanine, valine,

glutamine, histidine, or lysine residue,

a3 is lysine, tyrosine, arginine, glutamic acid, methionine, or

tryptophan residue,

a4 is glycine, alanine, valine, leucine, isoleucine, serine,

threonine, methionine, asparagine, glutamine,

histidine, lysine, arginine, phenylalanine, or

tryptophan residue

a5 is glycine, alanine, valine, leucine, isoleucine, serine,

threonine, methionine, asparagine, glutamine,

histidine, lysine, arginine, phenylalanine, tyrosine, or

tryptophan residue,

R is OH derived from carboxyl group or NH_2 derived from acid amide group).

Accordingly, the No.2 peptide in this invention is a 5 amino acid sequence that was constituted by a1, a2, a3, a4 and a5 as described above, and all of the peptide including such amino acid sequences contained by the range of this invention. Thus, a peptide having an affinity to gp120 represented by

Formula(5): a1'-a2-a3-a4-a5-R (SEQ ID No. 5),

(In the formula,

a1' means tyrosine, arginine, phenylalanine, glycine,

tryptophan, histidine, or aspartic residue ~~asparatic acid residue~~, or

polypeptide residue that an arbitrary amino acid stood

in the N-terminal side from this amino acid, a2, a3, a4,

a5 and R have the same meaning as above.)

or

Formula(6): H-a1-a2-a3-a4-a5' (SEQ ID No. 6),

(In the formula,

a5' is glycine, alanine, valine, leucine, isoleucine, serine, threonine, methionine, asparagine, glutamine, histidine, lysine, arginine, phenylalanine, tyrosine, or tryptophan residue, or polypeptide residue that an arbitrary amino acid stood in line in the C-terminal side of this amino acid, H, a1, a2, a3, and a4 ~~has~~ have the same meaning as above)

is entirely one aspect of the present invention.

In addition, this invention includes a compound which is a macromolecule compound that has a functional group and/or medicine bound to the No.1 or No.2 peptide as mentioned above, or a pharmaceutically acceptable salt thereof.

These peptide compounds or materials including them have an affinity to gp120.

Furthermore, the above peptides or a pharmaceutically acceptable salt thereof, and the composition including pharmaceutically acceptable carrier and/or medicinal bioactivity, are contained in this invention. Also, various aspects, such as; the detection, the diagnosis and the removal to viruses such as HIV by using the above peptide (for example, using the HIV diagnosis or the detection kit contained it, HIV absorbing and removal carrier, and therapy by plasmapheresis) are contained in this invention.

However, the above mentioned "peptide" that is used in this invention contained in the C-terminal peptide is COOH, acid amide and ester etc., and particularly, so long as we do not specify, it contains an ~~amino~~ amino acid number (~~origopeptide~~ oligopeptide) of less than 10, or a polypeptide of more than this.

An amino acid in the above mentioned peptide contains the derivatives that are protected by the protecting functional group. As such amino acid derivatives, it is

marketed by the substitution or the modification without exchanging the peptide structure; exchanging the length of the carbon chain etc., or the protecting amino acid derivatives corresponding to various amino acids, but all of these various amino acids can be used in this invention. For example, as tyrosine derivatives, there is 2,6-dichloro-L-tyrosine having chloride in the side chain, p-Nitro-L-phenylalanine that hydroxyl group of p-side in phenylalanine was substituted Nitroyl group, and 4-chloro-L-phenylalanine that the hydroxyl group was substituted chloride, etc. In addition, as valine derivatives, there are Norvaline: N- α -L-norvaline, or MeVal: N- α -L-valine, etc.

The reason that conventional medicine, such as a vaccine or neutrizing antibody can not be used clinically is that the HIV region the body can recognize as antigen is the V (hypervariable region) region in the envelop gp120, and this is the most problematic. So, the inventors researched a peptide which had a specific affinity to gp120, and as a result, developed a superior peptide and have applied for a patent already (Japanese Patent Application No. H 8-351474 and Japanese Patent Application No. H 8-351475). They developed a peptide which has a high specific affinity to gp120, of the same affinity or more compared to antibody , and which is additionally resistant to heat with a high pressure, such as in autoclave treatment.

However, from research after this, we found that the above-mentioned affinity to gp120 deteriorated by number and there was a kind of amino acid which ranged in it. So, we continued to research further to supply a more stable peptide, and make this invention complete.

In addition to the above, "affinity", this invention shows a specific and tight bond with weak interaction, such as electrostatic interaction, hydrogen bond, Van der Waals

attraction, hydrophobic bond and etc, gathered except a covalent bond.

The peptide in this invention is constituted as mentioned above and is fundamentally shown as 5 amino acid residues which appeared in;

①formula (1): H-A1-A2-A3-A4-A5-R (SEQ ID No.1),

(in formula, A1, A2, A3, A4, A5 and R, the meanings are the same as before) or,

②formula (4): H-a1-a2-a3-a4-a5-R (SEQ ID No. 4),

(in formula, a1, a2, a3, a4, a5 and R, the meanings are the same as before).

This peptide has a molecule separate in each and is not (or in peptide) space, the amino acid sequence mentioned above ①peptide,

formula (2): A1'-A2-A3-A4-A5; (SEQ ID. No. 2) or

formula (3): A1-A2-A3-A4-A5'- (SEQ ID No. 3)

or above mentioned ②peptide includes the sequence which lined up from N-terminus.

Formula (4): a1'-a2-a3-a4-a5; (SEQ ID. No. 5) or

Formula (5): a1 -a2-a3-a4-a5' (SEQ ID No. 6)

(in formula, A1', A2, A3, A4, A5', a1', a2, a3, a4, a5', the meanings are the same as before).

Of course, in A1'-A2'-A3'-A4'-A5' (SEQ ID Nos. 1-3) or a1'-a2'-a3'-a4'-a5' (SEQ ID Nos. 4-6), there includes peptides which lined up repeatedly by this order. In brief, this invention includes all of the peptides which consist of 5 amino acid residues and have an affinity to gp120.

The peptide in this invention can be synthesized by conventional methods; For example, the first of this invention is constituted from A1-A2-A3-A4-A5, is synthesized (SEQ ID No. 1)

and the A5 glycine residue, carboxyl of N-protective glycine is bound to some carrier, such as insoluble resin, which has a functional group that can couple to carboxyl. After this, the protected amino acid in each, from A2 to A5, is bound in order by a solid phase synthetic method, and the peptide shown in this invention can be obtained by reacting the above mentioned insoluble resin and eliminating the protection of the amino acid.

In addition to the above, an end of carboxyl in A5 amino acid residue is free(R, that is to say is equivalent to -OH), or is substituted with acidic amide(R, that is to say is equivalent to $-NH_2$). Then, an end-carboxyl of A5 with carboxyl of spacer together, bound this carboxyl, binds a synthetic macromolecule, bio-macromolecule, and utilized well macromolecular compound, which has a functional group (as in the postscript). In addition to the above, amino acid used by the above mentioned solid phase synthetic methods is common to L type or D type, but L type is more pleasing.

In the case of the above, the carrier used solid phase synthetic method has carboxyl group of N-protected glycine of C-terminus through the amino group, or if it can bind to this carboxyl group and can eliminate after the binding, it is not limited at all. For example, chloromethyl-resin (chloromethylpolystyrenedivinylbenzene), oxymethyl-resin (oxymethylpolystyrenedivinylbenzene) and others are exemplified. Then, resin of 4-(oxymethyl)phenylacetamidemethyl-resin, 4-(2',4'-Dimethoxyphenyl-aminomethyl)phenoxyacetamidemethyl-resin and etc., benzyloxybenzylalcohol-resin, benzhydrylamine-resin, insoluble-resin which has amino group, methylbenzhydrylamine-resin, aminomethylphenoxymethyl-resin, dimethoxybenzhydrylamine (DMBHA)-resin, and the derivatives are exemplified. In these, benzhydrylamine-resin, methylbenzhydrylamine-resin,

aminomethylphenoxymethyl-resin and DMBHA-resin can be get directly amide by cleavage after the binding. Judging from the yield, use of aminomethyl-resin is desirable.

In addition, a spacer which has a functional group binding with carboxyl group and has a carboxyl group are picked up which can transform p-carboxymethylbenzylester-resin to carboxyl group of glycine as in the example.

Moreover, "protecting amino acid" in the case of the above means protected amino acid with protecting group by conventional method. To synthesize the peptide invented, it is pleasing to use either of the protecting groups shown in the following examples.

In this example, the protecting group of α -amino in amino acid is Boc (t-butoxycarbonyl) or Fmoc (9-fluorenylmethoxycarbonyl); protecting group of ξ -amino in lysine is Z (benzyloxycarbonyl), Cl · Z(2-chlorobenzyloxycarbonyl), Boc, Npys(3-nitro-2-pyridinesulfonyl); protecting group of hydroxyl group in tyrosine is Bzl(benzyl), Cl_2 · Bzl(2,6-dichlorobenzyle), or t-Bu(*tert.*-butyl) are exemplified, but it uses the peptide synthesis well even if the hydroxyl group of this tyrosine is not protected by the above mentioned protecting group; protecting group of guanidino group in arginine is Tos(tosyl), NO_2(Nitro), Mtr(4-methoxy-2,3,6-trimethylbenzenesulfonyl), or pmc(2,2,5,7,8-pentometylchloroman-6-sulfonyl); protecting group of carboxyl group in glutamic acid is Bzl ester, t-Bu ester, cHx (cyclohexlylester); protecting group of amide group in glutamine is Trt(trityl) is picked up, but it can be used even if glutamine is not protected by this protecting group; the protecting group of indole group in tryptophan is formyl group or Boc, but it can be used even if tryptophan is not protected by this

case using chloromethyl resin as insoluble resin, it is treated by hydrogen fluoride with anisole. Then, in this case used benzyloxybenzylalcohol-resine, benzhydrylamine-resin, and DMBHA resin(Funakoshi, S., J. Chem. Soc., Chem. Commun., 198: 382,1988) , this resin and protecting group can be eliminated at same time by treatment of hydrofluore, TFMSA (Trifluoromethanesulfonicacid), TMSOTF (Trimethylsilyltriflate), TMSBr (Trimethylsilylbromide) and others.

The peptide that is obtained in this way can be purified by various methods; chromatography (gel, ion-exchange, hydrophobicity, adsorption, reverse phase), electrophoresis and ultrafiltration.

Also included in this invention is a peptide that was substituted on a similar protein(active center or binding domain of antibody, receptor, enzyme and etc.) by a gene recombination method in the case of above peptide. For example, if we produce human anti-gp120 antibody by gene recombinant method, we produce the above-mentioned peptide which is based on the U.S. Patent No. 114632. Namely, this peptide is transduced amino acids of hypervariable cluster in CDR (complementarity determination region, VH31 to VH35)-1 and CDR-2(VH50 to 52, and/or VH58 to 60), which relates recognition of epitope during the V region in the human immunoglobin gene (Ohno, S., Mori, N. & Matunaga, T.; Proc. Natl. Acad. Sci. USA. 82, 2945, 1985).

In this way, the peptide of this invention can produce specific binding to, gp120 accordingly to its purpose, substituting the gene recombinant method.

For example, the first peptide in this invention is shown from table 1 to 2 and at same the second peptide is shown from table 3 to 4, respectively. As in the case above,

TABLE 1

No.			A1	A2	A3	A4	A5	Agglutinin test	Neutralizing activity
1			Asp	Val	Lys	Ala	Gly	*	
2			Asp	Lys	Val	Ala	Gly	*	
3			Lys	Val	Asp	Ala	Gly	*	
4			Val	Lys	Lys	Ala	Gly		*
5			Asp	Asp	Lys	Ala	Gly		*
6			Lys	Asp	Asp	Ala	Gly	*	
7			Val	Asp	Asp	Ala	Gly	*	
8			Asp	Val	Asp	Ala	Gly	*	
9			Val	Val	Lys	Ala	Gly	*	*
10			Val	Val	Asp	Ala	Gly	*	
11			Lys	Val	Val	Ala	Gly	*	
12			Asp	Asp	Val	Ala	Gly	*	*
13			Asp	Asp	Asp	Ala	Gly		*
14			Val	Lys	Val	Ala	Gly		*
15			Asp	Tyr	Lys	Ala	Ala		*
16			Asp	Phe	Lys	Ala	Gly	*	
17			Asp	Trp	Lys	Ala	Gly	*	
18			Asp	Val	Arg	Ala	Gly	*	
19			Glu	Val	Lys	Ala	Gly	*	
20	Gly	Gly	Asp	Val	Lys	Ala	Gly	*	
21		Gly	Asp	Val	Lys	Ala	Gly	*	
22			Val	Ile	Asp	Ala	Gly	*	
23			Val	Leu	Asp	Ala	Gly	*	
24			Gly	Val	Lys	Ala	Gly	*	
25			Asp	Val	Lys	Trp	Ala	*	
26			Asp	Val	Lys	Gly	Lys	*	
27			Asp	Val	Lys	Gly	Trp	*	

Nos. 1-19 and 22-27 of Table 1 correspond to SEQ ID No. 1
No. 20 of Table 1 corresponds to SEQ ID No. 7
No. 21 of Table 1 corresponds to SEQ ID No 8.

TABLE 2

No.			A1	A2	A3	A4	A5	agglutinin test	neutralizing activity
28			Asp	Val	Ala	Ala	Gly	*	
29			Asp	Val	Lys	Gly	Leu	*	
30			Asp	Val	Lys	Gly	Pro	*	
31			Asp	Val	Lys	Ala	Val	*	
32			Asp	Val	Lys	Ala	Ile	*	
33			Asp	Val	Lys	Ala	Ser	*	
34			Asp	Val	Lys	Ala	Thr	*	
35			Asp	Val	Lys	Ala	Met	*	
36			Asp	Val	Lys	Ala	Gln	*	
37			Asp	Val	Lys	Ala	Asn	*	
38			Asp	Val	Lys	Ala	His	*	
39			Asp	Val	Lys	Ala	Arg	*	
40			Asp	Val	Lys	Ala	Phe	*	

Nos. 28-40 of Table 2 correspond to SEQ ID No. 1.

Changes is incorporated in the substitute page

TABLE 3

No.			a1 ~~A1~~	a2 ~~A2~~	a3 ~~A3~~	a4 ~~A4~~	a5 ~~A5~~	Agglutinin test	Neutralizing activity
1			Phe	Tyr	Arg	Lys	Ala	*	*
2			Tyr	Arg	Arg	Ala	Ala		*
3			Trp	Trp	Glu	Ala	Ala	*	
4			Tyr	Gln	Glu	Ala	Ala	*	*
5			Gly	Tyr	Tyr	Lys	Ala	*	*
6			Trp	Trp	Lys	Ala	Ala		*
7			Tyr	Tyr	Arg	Ala	Ala		*
8			Phe	Arg	Lys	Ala	Ala	*	*
9			Tyr	Tyr	Lys	Lys	Ala		*
10			Tyr	Tyr	Lys	Leu	Leu	*	*
11			Tyr	Arg	Lys	Ala	Ala	*	*
12			Tyr	Tyr	Lys	Ala	Ala	*	*
13			Arg	Tyr	Lys	Ala	Ala		*
14			Phe	Tyr	Arg	Ala	Ala	*	*
15			Tyr	Ala	Lys	Ala	Ala	*	
16			Tyr	Tyr	Glu	Ala	Ala	*	
17			Tyr	Trp	Lys	Ala	Ala	*	
18		Gly	Tyr	Tyr	Lys	Ala	Ala	*	
19			Trp	Tyr	Lys	Ala	Ala	*	
20			Tyr	Gln	Lys	Ala	Ala	*	
21			His	Tyr	Lys	Ala	Ala	*	*
22			Tyr	Arg	Tyr	Ala	Ala		*
23			Tyr	Tyr	Met	Ala	Ala		*
24			Tyr	Val	Lys	Ala	Ala	*	
25		Gly	Tyr	Ala	Tyr	Arg	Lys	*	*
26			Arg	Arg	Trp	Ala	Tyr	*	
27		Arg	Tyr	Tyr	Lys	Ala	Ala		

Nos. 1-17, 19-24 and 26 of Table 3 correspond to SEQ
No. 18 of Table 3 corresponds to SEQ ID No. 9.
No. 25 of Table 3 corresponds to SEQ ID No. 10.
No. 27 of Table 3 corresponds to SEQ ID No. 11.

Changes as incorporated in substitute page

TABLE 4

No.			a1 ~~A1~~	a2 ~~A2~~	a3 ~~A3~~	a4 ~~A4~~	a5 ~~A5~~	Agglutinin test	Neutralizing activity
28			Tyr	Lys	Lys	Ala	Ala	*	
29			Tyr	His	Lys	Ala	Ala	*	*
30			Asp	Tyr	Lys	Ala	Ala	*	
31			Tyr	Tyr	Lys	Trp	Ala	*	
32			Tyr	Tyr	Lys	Gly	Ala	*	
33			Tyr	Tyr	Lys	Ala	Gly	*	
34			Tyr	Tyr	Lys	Lys	Ala	*	
35			Tyr	Tyr	Lys	Val	Ala	*	
36			Tyr	Tyr	Lys	Ile	Ala	*	
37			Tyr	Tyr	Lys	Ser	Ala	*	
38			Tyr	Tyr	Lys	Thr	Ala	*	
39			Tyr	Tyr	Lys	Met	Ala	*	
40			Tyr	Tyr	Lys	Gln	Ala	*	
41			Tyr	Tyr	Lys	Asn	Ala	*	
42			Tyr	Tyr	Lys	His	Ala	*	
43			Tyr	Tyr	Lys	Phe	Ala	*	
44			Tyr	Tyr	Lys	Trp	Ala	*	
45			Tyr	Tyr	Lys	Arg	Ala	*	
46			Tyr	Tyr	Lys	Ala	Val	*	
47			Tyr	Tyr	Lys	Ala	Ile	*	
48			Tyr	Tyr	Lys	Ala	Ser	*	
49			Tyr	Tyr	Lys	Ala	Thr	*	
50			Tyr	Tyr	Lys	Ala	Met	*	
51			Tyr	Tyr	Lys	Ala	Gln	*	
52			Tyr	Tyr	Lys	Ala	Asn	*	
53			Tyr	Tyr	Lys	Ala	His	*	
54			Tyr	Tyr	Lys	Ala	Phe	*	
55			Tyr	Tyr	Lys	Ala	Trp	*	
56			Tyr	Tyr	Lys	Ala	Arg	*	

Nos. 28-56 of Table 4 correspond to SEQ ID No. 4.

A sign of each amino acid formula shows the amino acid residue by the internationally approved characters, the details are as follows:

Tyr: Tyrosine

Lys: Lysine

Trp: Tryptophan

Arg: Arginine

Changes as incorporated in the substitute page.

Glu: Glutamic acid

Gln: Glutamine

His: Histidine

Ala: Alanine

Phe: Phenylalanine

Gly: Glycine

Met: Methionine

Asp: ~~Asparatic~~ Aspartic acid

Asn: Asparagine

Val: Valine

Ser: Serine

Cys: ~~Cystine~~ Cysteine

Thr: Threonine

Ile: Isoleucine

Leu: Leucine

Pro: Proline

A peptide having such an amino acid sequence shows a superior affinity to gp120, and can be utilized effectively as an anti-HIV medicine by taking a form of chemical compound or composition shown as follows.

A compound of this invention is matter that binds a high molecular chemical compound and/or medicinal activator functional group, and this invention includes the salts to be admitted as medicine.

For example, as pharmaceutically acceptable salts here, following intoxicant salts in

common use is put up.

①As salts with bases such as inorganic bases, there are alkali metal salt (for example, sodium salt and potassium salt), alkaline earth metal salt (for example, calcium salt, magnesium salt) and ammonium salt; ② as salts with such as organic bases, there is salts of organic amines (for example, triethylamine, pyridine, picoline, ethanolamine, triethanolamine, dicyclohexylamine, N,N'-dibenzylethylenediamines), etc; ③ as salts with acids such as inorganic acids, there are hydrocholic acid, hydrobromic acid, phosphoric acid, nitric acid, sulfuric acid; ④ as salts with acids such as organic acids, there are organic carboxylic acid (for example, acetic acid, propionic acid, maleic acid, succinic acid, malic acid, citric acid, tartaric acid and salicylic acid), organic sulfonic acid (for example, methanesulfonic acid, p-toluenesulfonic acid), glyconic acid (glucuronic acid, galactonic acid, gluconic acid, ascorbic acid , and others).

Then, "macromolecule compound having functional group" used on this invention is not particularly limited if it can bind the peptide of this invention, for example, the following are listed.

(1) Synthetic polymer

It is selected voluntarily among the inside of linear, branched and cyclic, as polymer in case of above. For example, it can be used as an insoluble solid phase carrier of amino acidic homopolymer of polylysine and polyglutamic acids, or cyclic ~~polyaimne~~ polyamine, cyclodextrin, cyclic peptide and then polystylene, polypropyrene, nylon, silica-gel, polyethyleneglycol, cellulose, polyacrylamide, and others.

A branched polymer in these is higher than the usual homopolymer on content of functional groups per unit because of divergence at one part in each. For example, it seems to be the lysine core indicated by Denkewalter and is a polymer that is based on the same molecular chain, more than two, derived from a core molecule having at least two or more having functional groups U.S. Patent No. 4,289,872; or it is a starburst dendrimer that the polymer size is regulated closely because the same molecule reacts continuously, proposed by Tomalia and et al; or it is the molecule that size was formed irregular , by which the same or different molecule reacts to discontinuity. In addition, a homo-/branched-polymer as in the case of above does not always need a carrier which has enough size, and it has a monomer of around 3 that does not usually seem to be recognized as a core, and it is not limited by the size or introduction number. However, in the case when it is introduced to numerous peptide formulas, use of divergence numerical polymer is recommended but even if it is anything polymer. When the peptide of this invention is bound (to the above mentioned polymer) it is possible that it is synthesized and just grown directly/indirectly from the branched functional group, or that it is conjugated directly/indirectly from the functional group of the polymer to a new separate synthetic peptide.

Moreover, for binding cyclic polymer of cyclic polyamine, cyclodextrin, and cyclic peptides, it is possible to ~~synthesis~~ synthesize directly and make the peptide of upper expression from the same functional group, or to bind directly/indirectly to a new synthetic peptide separately or to a functional group of the cyclic polymer. Then, to bind the insoluble carrier of silica gel etc., after it is introduced to the same functional group carrier in advance, it can be synthesized and just grow directly peptide of upper expression from

the functional group, or conjugate directly/indirectly from the functional group of insoluble carrier to the new synthetic peptide separately. In addition, the particular size and shape of the carrier having this same functional group is not limited, and selection and utilization of: spherical, hollow fibrous, fibrous shapes can be made according to their purpose and then, it is not limited at all by size and shape and can be introduced to several functional groups.

(2) Biopolymer

As above mentioned biopolymer, there are, for example, linear polymer like polysaccharide, such as heparin, hyaluronic acid, chitosan, chitin, etc.; proteins of proteoglycans, peptide hormone; gelatin, albumin, antibody, and antibody's fragments, etc.

The size of linear polymer in these can be an appropriately selected, according to the purpose of use, and includes some monomer of around 3 that does not usually seems to be recognized as a polymer, but is not limited at all by the size or number of functional groups. For binding a peptide of upper expression to this linear polymer, you may directly synthesize and just grow it from the same functional group, or may directly/indirectly conjugate a new synthetic peptide separately to the functional group of the linear polymer.

In addition, when peptide hormone and protein is bound to the polymer, it is possible that either end of the peptide of upper expression is bound to cysteine and do disulfide binding with the residue of cysteine in the above mentioned peptide hormone/protein, or

to C-terminus of cysteine of No.12 peptide on Table 3 mentioned above, it reacted to the above maleimide cyclodextrin, and we synthesized a cyclic compound that cyclodextrin binding compound to the peptide.

Synthesis 3: Compound (1) which was bound branched polymer with the peptide in this invention

Branched polymer binding compound was synthesized by extending No.1 peptide on the above Table 1 from N-end amino acid MAPs (Multiple antigenic peptide). After the compound was suspended in phosphate buffer, it was purified by gel chromatography and affinity chromatography by gp120 conjugated carrier, and then, we synthesized branched polymer binding compound to the peptide in this invention (1).

Synthesis 4: Compound (2) which was bound branched polymer with the peptide in this invention

Branched polymer binding compound was synthesized by extending No.12 peptide on the above Table 3 from N-end amino acid MAPs (Multiple antigenic peptide). After the compound was suspended in phosphate buffer, it was purified ~~by~~ through gel chromatography and affinity chromatography by gp120 conjugated carrier, and we synthesized branched polymer binding compound to the peptide in this invention (2).

After incubation for 30 minutes at 37℃, 100 μL of MT-4 cell suspension of 3×10^4 cells was added and incubated for 6 days at 37℃ under 98% of humidity and 5% of CO_2. After the incubation, cell plasmodium effect (CPE) by HIV-1 prolification, namely when a medicine was added with dilution step-by-step and infection cells gathered and became a state to form an island(focus form), was judged as an intensity of neutralizing activity(content to inhibit infection) on a pre-stage of this diluted magnification. These results are shown jointly in Tables 5 and 6.

TABLE 5

	Amino acid sequence					HIV neutralizing activity(μg/ml)	
No.	A1	A2	A3	A4	A5	HTLV-IIIB	KK-1
1	Val	Lys	Lys	Ala	Gly	Nglu	15.6
2	Asp	Asp	Lys	Ala	Gly	62.5	NE
3	Val	Val	Lys	Ala	Gly	31.3	NE
4	Asp	Asp	Val	Ala	Gly	500	NE
5	Asp	Asp	Asp	Ala	Gly	1,000	1,000
6	Val	Lys	Val	Ala	Gly	125	NE
7	Asp	Tyr	Lys	Ala	Ala	31.3	125

Nos. 1-7 of Table 5 correspond to SEQ ID No. 1.

TABLE 6

	Amino acid sequence					Anti-HIV neutralizing activity(μ g/ml)	
No.	a1	a2	a3	a4	a5	HTLV-IIIB	KK-1
1	Phe	Tyr	Arg	Lys	Ala	250	125
2	Tyr	Arg	Arg	Ala	Ala	250	125
3	Trp	Trp	Glu	Ala	Ala	250	NE
4	Gly	Tyr	Tyr	Lys	Ala	31.3	125
5	Tyr	Tyr	Arg	Ala	Ala	250	250
6	Phe	Arg	Lys	Ala	Ala	125	125
7	Tyr	Tyr	Lys	Lys	Ala	312.5	312.5
8	Tyr	Tyr	Lys	Leu	Leu	31.3	62.5
9	Tyr	Arg	Lys	Ala	Ala	312.5	156.3
10	Tyr	Tyr	Lys	Ala	Ala	78.1	39.1
11	Arg	Tyr	Lys	Ala	Ala	62.5	31.25
12	Phe	Tyr	Arg	Ala	Ala	NE	250-125
13	Tyr	Ala	Lys	Ala	Ala	500	NE
14	His	Tyr	Lys	Ala	Ala	NE	500
15	Tyr	Arg	Tyr	Ala	Ala	31.25	31.25
16	Tyr	Tyr	Met	Ala	Ala	NE	125
17	Tyr	Val	Lys	Ala	Ala	NE	250
18	Arg	Arg	Trp	Ala	Tyr	39.1	39.1
19	Tyr	His	Lys	Ala	Ala	500	500

Nos. 1-19 of Table 6 correspond to SEQ ID No. 4.

Note) HTLV-IIIB; Laboratory strain

KK-1; Freshly isolated strain from domestic HIV patients

NE; No effect

As is clear from the result of Tables 5 and 6, neither peptide which was not satisfied with a matter of this invention showed neutralization activity to HIV-1, while the peptides which satisfied it showed superior activity. Then, a peptide of this invention shown in Table 5 presents No.1 peptide of this invention, and the peptide of Table 6 shows the No.2, in each. In addition to the laboratory strain, when this invented peptide showed superior activity to the freshly isolated strain, this suggested it would be

TABLE 7

No.	Amino acid sequence							Agglutinin test gp120/C.G.
			A1	A2	A3	A4	A5	
1			Asp	Val	Lys	Ala	Gly	+++
2			Asp	Lys	Val	Ala	Gly	+
3			Lys	Val	Asp	Ala	Gly	+++
4			Asp	Asp	Lys	Ala	Gly	+
5			Lys	Asp	Asp	Ala	Gly	++
6			Val	Asp	Asp	Ala	Gly	++
7			Asp	Val	Asp	Ala	Gly	+
8			Val	Val	Lys	Ala	Gly	+
9			Val	Val	Asp	Ala	Gly	+++
10			Lys	Val	Val	Ala	Gly	+
11			Asp	Tyr	Lys	Ala	Ala	+
12			Asp	Phe	Lys	Ala	Gly	++
13			Asp	Trp	Lys	Ala	Gly	++
14			Asp	Val	Arg	Ala	Gly	+
15			Glu	Val	Lys	Ala	Gly	++
16	Gly	Gly	Asp	Val	Lys	Ala	Gly	+++
17		Gly	Asp	Val	Lys	Ala	Gly	++
18			Val	Ile	Asp	Ala	Gly	+
19			Val	Leu	Asp	Ala	Gly	+
20			Gly	Val	Lys	Ala	Gly	+
21			Asn	Val	Lys	Ala	Gly	+++
22			Asp	Val	Lys	Trp	Ala	+
23			Asp	Val	Lys	Gly	Lys	+
24			Asp	Val	Lys	Gly	Trp	+
25			Asp	Val	Lys	Gly	Leu	+
26			Asp	Val	Lys	Gly	Pro	+

Nos. 1-15 and 18-26 of Table 7 correspond to SEQ ID No. 1.
No 16 of Table 7 corresponds to SEQ ID No 7
No 17 of Table 7 corresponds to SEQ ID No. 8.

TABLE 8

No.	Amino acid sequence							Agglutinin test gp120/C.G.
			A1	A2	A3	A4	A5	
27			Asp	Val	Lys	Ala	Val	+
28			Asp	Val	Lys	Ala	Ile	+
29			Asp	Val	Lys	Ala	Ser	+
30			Asp	Val	Lys	Ala	Thr	+
31			Asp	Val	Lys	Ala	Met	+
32			Asp	Val	Lys	Ala	Gln	+
33			Asp	Val	Lys	Ala	Asn	+
34			Asp	Val	Lys	Ala	His	+
35			Asp	Val	Lys	Ala	Arg	+
36			Asp	Val	Lys	Ala	Phe	+

Nos. 27-36 of Table 8 correspond to SEQ ID No. 1.

TABLE 9

No.	Amino acid sequence						Agglutinin test gp120/C.G.
		a1	a2	a3	a4	a5	
1		Phe	Tyr	Arg	Lys	Ala	+
2		Trp	Trp	Glu	Ala	Ala	+
3		Tyr	Gln	Glu	Ala	Ala	+
4		Gly	Tyr	Tyr	Lys	Ala	+
5		Trp	Trp	Lys	Ala	Ala	+++
6		Tyr	Tyr	Lys	Lys	Ala	+
7		Tyr	Arg	Lys	Ala	Ala	+
8		Tyr	Tyr	Lys	Ala	Ala	+
9		Arg	Tyr	Lys	Ala	Ala	+
10		Tyr	Ala	Lys	Ala	Ala	+
11		Tyr	Tyr	Glu	Ala	Ala	+
12		Tyr	Trp	Lys	Ala	Ala	+
13	Gly	Tyr	Tyr	Lys	Ala	Ala	+
14		Trp	Tyr	Lys	Ala	Ala	+
15		Tyr	Gln	Lys	Ala	Ala	++
16		His	Tyr	Lys	Ala	Ala	+
17		Tyr	Arg	Tyr	Ala	Ala	++
18	Gly	Tyr	Ala	Tyr	Arg	Lys	+
19		Arg	Arg	Trp	Ala	Tyr	+
20	Arg	Tyr	Tyr	Lys	Ala	Ala	+
21		Tyr	Lys	Lys	Ala	Ala	+
22		Tyr	His	Lys	Ala	Ala	+
23		Asp	Tyr	Lys	Ala	Ala	+
24		Tyr	Tyr	Lys	Trp	Ala	+
25		Tyr	Tyr	Lys	Gly	Ala	+
26		Tyr	Tyr	Lys	Ala	Gly	+

Nos. 1-12, 14-17 19 and 21-26 of Table 9 correspond to SEQ ID No4
No. 13 of Table 9 corresponds to SEQ ID No. 9.
No. 18 of Table 9 corresponds to SEQ ID No. 10
No. 20 of Table 9 corresponds to SEQ ID No. 11.

Changes as incorporated in the substitute page

TABLE 10

	Amino acid sequence						Agglutinin test
No.		a1	a2	A3a3	a4	a5	gp120/C.G.
27		Tyr	Tyr	Lys	Lys	Ala	+
28		Tyr	Tyr	Lys	Val	Ala	+
29		Tyr	Tyr	Lys	Ile	Ala	+
30		Tyr	Tyr	Lys	Ser	Ala	+
31		Tyr	Tyr	Lys	Thr	Ala	+
32		Tyr	Tyr	Lys	Met	Ala	+
33		Tyr	Tyr	Lys	Gln	Ala	+
34		Tyr	Tyr	Lys	Asn	Ala	+
35		Tyr	Tyr	Lys	His	Ala	+
36		Tyr	Tyr	Lys	Phe	Ala	+
37		Tyr	Tyr	Lys	Trp	Ala	+
38		Tyr	Tyr	Lys	Arg	Ala	+
39		Tyr	Tyr	Lys	Ala	Val	+
40		Tyr	Tyr	Lys	Ala	Ile	+
41		Tyr	Tyr	Lys	Ala	Ser	+
42		Tyr	Tyr	Lys	Ala	Thr	+
43		Tyr	Tyr	Lys	Ala	Met	+
44		Tyr	Tyr	Lys	Ala	Gln	+
45		Tyr	Tyr	Lys	Ala	Asn	+
46		Tyr	Tyr	Lys	Ala	His	+
47		Tyr	Tyr	Lys	Ala	Phe	+
48		Tyr	Tyr	Lys	Ala	Trp	+
49		Tyr	Tyr	Lys	Ala	Arg	+

Nos 27-49 of Table 10 correspond to SEQ ID No 4.

Note) gp120/C.G.; gp120 conjugated colloidal gold

Degree of agglutinination: +++ > ++ > +(agglutinination), -(no agglutinination)

From the results in Tables 7-10, we can confirm that each peptide shown in this invention has a superior affinity to gp120. The peptide of this invention is shown in Tables 7 and 8 is the No.1 peptide in this invention, and it shown in Tables 9 and 10 is the No.2 peptide of it.

EXAMPLE 3: Effect of A4 and A5

in this invention on the agglutinin test

To examine the effect of A4 and A5 amino acid on the aggluitinin test, the chain length of the No.1 peptide in TABLE 7 was changed. The agglutinination was measured the same as in 2. These results are shown jointly in TABLE 11. Face note, ± means "agglutinination in trace degree".

TABLE 11

	Amino acid sequence					Agglutinin test
No.	A1	A2	A3	A4	A5	gp120/C.G.
1	Asp	Val	Lys	Ala	Gly	+++
2	Asp	Val	Lys	Ala	-	+
3	Asp	Val	Lys	-	-	±

No.1 of Table 11 corresponds to SEQ ID No 1.
No.2 of Table 11 corresponds to SEQ ID. No.12
No.3 of Table 11 corresponds to SEQ ID No 13

A4 A5

From TABLE 11, both No.3 with only 3 amino acids (there are no ~~a4~~ and ~~a5~~ amino acids) and in No.2 with only 4 amino acids(there are no a5 amino acids) neutralizing activity decreased remarkably ~~composed~~ compared to No.1 with five amino acids. Namely, by the amino acid numerical decrease, the neutralizing activity has a tendency to fall, and particularly No.3, having only three amino acids ~~missing in all~~.

EXAMPLE 4: Effect of a4 and a5

in this invention on neutralizing activity

To examine the effect of a4 and a5 amino acids on neutralizing activity, the chain length of No.8 in ~~table~~ Table 9 were changed. The activity was measured the same as in

Changes as incorporated in the substitute page

example 1. Their results are shown in TABLE12. Face note, NE means "NO EFFECT".

TABLE 12

	Amino acid sequence					Anti-HIV neutralizing activity (μg/ml)	
No.	a1	a2	a3	a4	a5	HTLV-IIIB	KK-1
1	Tyr	Tyr	Lys	Ala	Ala	78.1	39.1
2	Tyr	Tyr	Lys	Ala	-	NE	250
3	Tyr	Tyr	Lys	-	-	NE	NE

No. 1 of Table 12 corresponds to SEQ ID No. 4
No. 2 of Table 12 corresponds to SEQ ID No. 14
No. 3 of Table 12 corresponds to SEQ ID No. 15

From TABLE12, both No.3 with only 3 amino acids(there is not a4 and a5 amino acid) and No.2 with only 4 amino acids(there is not a5 amino acid) neutralizing activity decreased remarkably compared to No.1 with five amino acids. Namely, by amino acid numerical decrease, the neutraizing activity has a tendency to fall, and particularly, No.3 having three amino acids missing in all.

EXAMPLE 5: Effect of a4 and a5
in this invention on neutralizing activity

To examine the effect of a4 and a5 on neutralizing activity, No.1 peptide in TABLE 13 was used as a positive control, while we used a peptide(No.2) that kind of a4 amino acids was changed to leucine, hydrophobic amino acid of same as alanine , or a peptide(No.3) that was changed to proline. Then, we examined the effect to neutralizing activity as in EXAMPLE 1. The results are shown jointly in TABLE 13.

TABLE 13

	Amino acid sequence					Anti-HIV neutralizing activity (μg/ml)	
No.	a1	a2	a3	a4	a5	HTLV-IIIB	KK-1
1	Tyr	Tyr	Lys	Ala	Ala	78.1	39.1
2	Tyr	Tyr	Lys	Leu	Leu	31.3	62.5
3	Tyr	Tyr	Lys	Pro	Pro	NE	NE

Nos 1-2 of Table 13 correspond to SEQ ID No. 4.
No. 3 of Table 13 corresponds to SEQ ID No. 16.

From TABLE 13, the kinds of a4 and a5 amino acids differed from the amino acid that was identified by this invention on No.1, the neutralizing activity decreased or disappeared. The results suggest that these kinds of a4 and a5 amino acid have an important effect on the expression of the activity.

EXAMPLE 6: Effect of A4 and A5

in this invention on ~~aglutinin~~ agglutinin test

We examined the effect of A4 and A5 on agglutinin test as shown in TABLE 14. The No.1 peptide was used as a positive control, while we used a peptide (No.2) that kind of A4 amino acids was changed to leucine, hydrophobic amino acid of same as alanine , or a peptide(No.3) that was changed to aspartic acid, acidic amino acid; similarly, the peptide(No.4) was changed to proline, and the peptide(No.5) was changed to glutamic acid. Then, we examined the effects on neutralizing activity the same as in EXAMPLE 2. The results are shown jointly in ~~table~~ Table 14. Face note, ± means "an agglutinin in trace degree".

TABLE 14

	Amino acid sequence					Agglutinin test
No.	A1	A2	A3	A4	A5	Colloidal gold conjugated gp120
1	Asp	Val	Lys	Ala	Gly	+++
2	Asp	Val	Lys	Pro	Gly	-
3	Asp	Val	Lys	Asp	Gly	-
4	Asp	Val	Lys	Ala	Pro	-
5	Asp	Val	Lys	Ala	Glu	±

Nos. 1 and 4 of Table 14 correspond to SEQ ID No. 1
No. 2 of Table 14 corresponds to SEQ ID. No. 17
No. 3 of Table 14 corresponds to SEQ ID No. 18. No. 5 of Table 14 corresponds to SEQ ID No. 1

From TABLE 14, when a kind of A4 and A5 amino acid on No.1 were exchanged to a different amino acid, the agglutinin was negative, or decreased to a trace degree. These results suggest that the kind of A4 and A5 amino acids have important effects on agglutinin test.

EXAMPLE 7: Effect of macromolecularization on neutralizing activity

We examined the effect of the peptide binding macromolecule on neutralizing activity. An inactive alkaline phosphatase (Alp) was used as the macromolecule, and it was bound to the peptide that was prepared in SYNTHESIS 6, the same as in EXAMPLE 1. No.8 peptide was used as a positive control on TABLE 3, while unbound inactive alkaline phophatase was used as negative control. These results are shown jointly in TABLE 15. Face note, NE means "negative".

EXAMPLE 9: the affinity of the peptide to gp120(1)

The peptide bound to Sephadex 6MB that was prepared in SYNTHESIS 7 was added to the various kinds of density prepared horseradish peroxidase (HRP) labeled HIV-1-gp120(Immuno Diagnosis Co.) and enzyme unlabeled HIV-1-gp120 previously in each and constant disassociation(kd) of peptide in this invention ~~caliculated~~ calculated by drawing up a Schacherd Plot we calculated was kd = 2.14×10^{-10}M.

From this result, it is clear that the peptide in this invention has an affinity of equal to, or greater than the antibody.

EXAMPLE 10: an affinity of the peptide to gp120(2)

The peptide binding Sephadex 4B that was prepared in SYNTHESIS 7 was added to the various kinds of density prepared horseradish peroxidase (HRP) and labeled HIV-1-gp120(Immuno Diagnosis Co.) and enzyme unlabeled HIV-1-gp120 previously in each and constant disassociation(kd) of the peptide in this invention calculated by drawing up a Schacherd Plot we calculated was kd = 4.97×10^{-10}M.

From this result, it is clear that the peptide in this invention has an affinity of equal to, or greater than the antibody.

EXAMPLE 11: a number of recognition site

of the peptide to gp120(1)

dilute virus solution of this degree, we measured the p24 content in this example and calculated S/CO by comparing it with other data.

EXAMPLE 15: the binding of HIV to latex conjugated peptide

We filmed the agglutination condition of HIV by No.2 virus/No.1peptide-latex beads in TABLE 17. In detail, after the HIV-1LAV1 laboratory strain was added to the above aggulutinin test medicine, it stood for 6 hours at 4℃. Afterwards, this agglutinated solution was put on a support membrane for observation by electron microscope, it was negatively stained with uranyl acetate and a photograph was taken. Referentially, we show this photograph in Fig. 2

From Fig. 2, we can observe that the HIV virus binds tightly to the latex beads conjugated HIV-gp120 affinity peptide, those beads bind mutually and agglutinate.

~~INDUSTRIAL APPLICABILITY~~

As described above, the peptide in this invention is; superior in stability, an affinity to gp120, is extremely useful as a medicine to treat HIV as it has neutralizing activity equal to previous antibody molecules, can be used as to diagnose HIV through agglutination and is a medical tool for removing HIV. It has physical stability and is resistant to autoclave treatment, which is not so of antibody molecules.

WHAT IS CLAIMED IS:

1. A peptide having affinity to gp120 represented by formula (1): H-A1-A2-A3-A4-A5-R (SEQ ID No. 1)

wherein, in the formula,

H means hydrogen,

A1 is aspartic acid, lysine, valine, glutamic acid, glycine, asparagine, or tyrosine residue,

A2 is valine, aspartic acid, tryptophan, lysine, phenylalanine, isoleucine, leucine, or tyrosine residue,

A3 is lysine, valine, aspartic acid, arginine, alanine, or tryptophan residue,

A4 is alanine, tryptophan, or glycine residue,

A5 is glycine, alanine, valine, leucine, isoleucine, serine, threonine, methionine, asparagine, glutamine, histidine, lysine, arginine, phenylalanine, tryptophan, proline, or tyrosine residue, and

R is OH derived from carboxyl group or NH_2 derived from acid amide group.

2. A peptide having affinity to gp120 represented by formula (2): A1'-A2-A3-A4-A5-R (SEQ ID No. 2)

wherein, in the formula,

A1' means aspartic acid, lysine, valine , glutamic acid, glycine, ~~aspragine~~ asparagine, or tyrosine residue, or polypeptide residue that an arbitrary amino acid stood in line in N-terminal side from this amino acid, and

A2, A3, A4, A5 and R ~~has~~ have the same meanings as ~~the above~~ in claim 1.

3. A peptide having affinity to gp120 represented by formula (3):

H-A1-A2-A3-A4-A5'-R^X (SEQ ID No. 3)

wherein, (in the formula,

A5' means glycine, alanine, valine, leucine, isoleucine, serine, threonine, methionine, asparagine, glutamine, histidine, lysine, arginine, phenylalanine, tryptophan, proline, or tyrosine residue, or polypeptide residue that an arbitrary amino acid stood in line in C-terminal side from this amino acid, and

H, A1, A2, A3 and A4 ~~is~~ have the same meaning as ~~the above~~ in claim 1.

4. A peptide having affinity to gp120 ~~characterized in having~~ comprising the amino acid sequence of

A1-A2-A3-A4-A5^F (SEQ ID No. 1).

5. A peptide having affinity to gp120 represented by Formula (4): H-a1-a2-a3-a4-a5-R (SEQ ID No. 4)

wherein (in the formula,

H means hydrogen,

a1 is tyrosine, arginine, phenylalanine, glycine, tryptophan, histidine, or ~~asparatic~~ aspartic acid residue,

a2 is arginine, tyrosine, tryptophan, alanine, valine, glutamine, histidine, or lysine residue,

a3 is lysine, tyrosine, arginine, glutamic acid, methionine, or tryptophan residue,

a4 is glycine, alanine, valine, leucine, isoleucine, serine, threonine, methionine, asparagine, glutamine, histidine, lysine, arginine, phenylalanine, or tryptophan

residue,

a5 is glycine, alanine, valine, leucine, isoleucine, serine, threonine, methionine, asparagine, glutamine, histidine, lysine, arginine, phenylalanine, tyrosine, or tryptophan residue, and

R is OH derived from carboxyl group or NH_2 derived from acid amide group.

6. A peptide having affinity to gp120 represented by Formula (5): a1'-a2-a3-a4-a5-R (SEQ ID No. 5)

wherein, in the formula,

a1' means tyrosine, arginine, phenylalanine, glycine, tryptophan, histidine, or aspartic acid residue, or polypeptide residue that an arbitrary amino acid stood in line in N-terminal side from this amino acid, and

a2, a3, a4, a5 and R have the same meaning as in claims.

7. A peptide having affinity to gp120 represented by Formula (6): H-a1-a2-a3-a4-a5' (SEQ ID No. 6)

wherein, in the formula,

a5' is glycine, alanine, valine, leucine, isoleucine, serine, threonine, methionine, asparagine, glutamine, histidine, lysine, arginine, phenylalanine, tyrosine, or tryptophan residue, or polypeptide residue that an arbitrary amino acid stood in line in C-terminal side from this amino acid, and

H, a1, a2, a3, and a4 have the same meaning as in claims.

8. A peptide having affinity to gp120 comprising the amino acid sequence of a1-a2-a3-a4-a5 (SEQ ID No. 4).

ABSTRACT OF THE DISCLOSURE

The peptide in this invention is a peptide having affinity to gp120 represented by Formula (1): H-A1-A2-A3-A4-A5-R (SEQ ID NO: 1)

(in the formula,

H means hydrogen,

A1 is aspartic acid, lysine, valine, glutamic acid, glycine, asparagine, or tyrosine residue,

A2 is valine, aspartic acid, tryptophan, lysine, phenylalanine, isoleucine, leucine, or tyrosine residue,

A3 is lysine, valine, aspartic acid, arginine, alanine, or tryptophan residue,

A4 is alanine, tryptophan, or glycine residue,

A5 is glycine, alanine, valine, leucine, isoleucine, serine, threonine, methionine, asparagine, glutamine, histidine, lysine, arginine, phenylalanine, tryptophan, proline, or tyrosine residue,

R is OH derived from carboxyl group or NH_2 derived from acid amide group).

The above peptide has an affinity to gp120 of the HIV envelope protein and is superior in stability.